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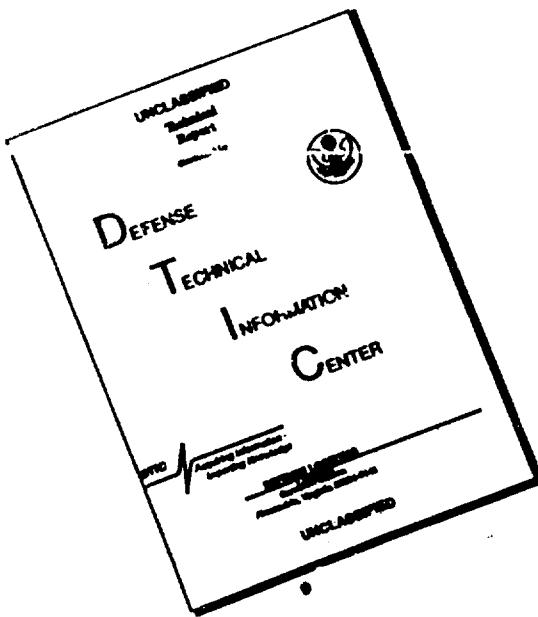
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IMMUNOGENICITY OF VARIOUS PHASES OF BURNET'S RICKETTSIAE

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According to the existing studies the immunogenicity of vaccines prepared from various phases of Burnet's rickettsiae is not the same (Fedorova and Dyuysaliyeva, 1963; Fedorova, 1964; Ormsbee et al, 1964, and others).

Ormsbee et al (1964) demonstrated that vaccines prepared from phase I Burnet rickettsiae are more immunogenic than those prepared from phase II. And the authors believe that the difference in immunogenicity is related to the phasic state rather than distinctions of the strains used to prepare the vaccine. There was negligible difference between immunogenicity of vaccines from different strains (Nine Mile, Ohio 314, California 16, Henzerling) (three to six-fold difference) whereas the vaccines prepared from different phases of the same strains presented 100 to 300-fold differences in immunogenicity.

The killed and live vaccine from attenuated strain M-44, used in the Soviet Union for the prevention of Q fever, is prepared from egg cultures of phase II Burnet rickettsiae. They contain very little or a negligible amount of phase I component (Vasil'yeva and Yablonskaya, 1955; Zdrodovskiy and Genig, 1962; Genig, 1960, 1963, 1965; Vorob'yev et al, 1965). And only the vaccine obtained from the spleens of albino mice at the Tashkent Institute of Vaccines and Sera (Khodukin et al, 1956, 1960) apparently is phase I vaccine. However it has not gained

wide approval for humans because of its reactogenicity. Vorob'yev et al (1965) proposed a soluble antigen prepared from phase I Burnet rickettsiae using trichloracetic acid as a chemical vaccine.

The purpose of the present study was to investigate the immunogenic properties of a vaccine in relation to phase variations of Burnet's rickettsiae. We used the standard Shorsher strain isolated from the blood of a patient in Yaroslavl' (Kulagin and Fedorova, 1957). The immunogenic properties of different phases of Burnet's rickettsiae were determined on guinea pigs according to the level of complement fixing antibodies in the blood and resistance following infection with a virulent strain of rickettsiae. Two series of corpuscular antigens prepared from yolk cultures of Burnet's rickettsiae were used as the vaccine following varying numbers of passages: 5th passage in series No 15, 60th passage in series No 17.

In order to identify the phase, the antigens were examined by means of checkerboard titration in the complement fixing reaction using the conventional technique. Guinea pig serum collected in the early (20th) and late (60th day) post-infection period following inoculation of a spleen culture (rickettsiae -- +++, ++++) served as the standard. The reaction was run under refrigeration with two units of antigen. Antigens and sera were used in the experiment in successive two-fold dilutions. The minimal dilution of antigen that elicited distinct delay of hemolysis (+++, ++++) was considered the antigen titer.

As shown in Table 1, with the antigen of series No 15, a positive complement fixing reaction was obtained only with serum collected at a late date. We classified it as phase I antigen. Series 17 antigen reacted in equal titers with sera collected both "early" and "late" i.e. it was phase II antigen.

For vaccination purposes we used antigens in the following concentrations: one billion, 250, 62, 31 and 16 million bacterial cells per milliliter. The concentration was determined by the bacterial standard and by nephelometry. Different concentrations of antigen were injected subcutaneously in a dosage of 0.5-1 milliliter twice at a 10-day interval to a group of guinea pigs weighing 250 to 300 grams (each concentration was administered to ten animals).

Blood samples were taken on the 30th, 60th and 80th post-vaccination day to run the complement fixing reaction with antigens from phase I and II Burnet rickettsiae. Table 2 shows that there were substantial differences between the serological indices of guinea pigs immunized with antigens of different phases. Thus, vaccination with phase I antigen elicited appearance of both "early" and "late" antibodies on the 30th day, their titer dropped somewhat by the 60th day then again rose following infection with a virulent phase I culture. Vaccination with phase II antigen elicited production only of "early" antibodies. "Late"

antibodies were demonstrable following infection with a virulent phase I culture, i.e. by the 80th postvaccination day. Some correlation was found between antibody titer and concentration of material injected. Thus, with immunization with antigen at a concentration of one billion and 250 million bacterial cells per milliliter, the antibody titer against phase II Burnet rickettsiae antigen on the 30th day constituted 1:640, at a concentration of 16 million it was 1:40 to 1:20.

Table 1  
Results of checkerboard titration of Burnet's rickettsiae  
(Shorsher strain) in the complement fixation reaction

		(A)		Титр антител с сыворотка- ми, полушен- ными в разные сроки после иммунизации		
		Антиген		(B)		
(C)	(d)	КОНЦЕНТРА- ЦИЯ ПО БАКТЕРИА- ЛЬНОМУ СТАН- ДАРТУ (в млн.)	(e)	(f)	(g)	(h)
I	5-A	1 000	1:2	—	512	
		500	1:4	—	512	
		250	1:8	—	512	
		125	1:16	—	256	
		62	1:32	—	128	
		31	1:64	—	32	
		16	1:128	—	—	
II	60-A	1 000	1:2	512	512	
		500	1:4	512	512	
		250	1:8	256	512	
		125	1:16	256	256	
		62	1:32	128	128	
		31	1:64	32	32	
		16	1:128	8	—	

Legend:

a) antigen	e) concentration according to bacterial standard (in millions)
b) antibody titer with sera obtained at different post- immunization intervals	f) dilution
c) phase	g) "early"
d) passage	h) "late"

Intensity of immunity was tested 60 days after vaccination by means of intraperitoneal injection of 100,000 ID [infective doses] of standard Burnet rickettsiae cultures. As the phase I culture we used a suspension of rickettsiae from the spleens of albino mice; as the phase II culture we used an egg culture (40th passage). The minimum infective dose was determined through titration tests on guinea pigs according to temperature

indices (a temperature above 39.5° was considered elevated).

Table 2  
Arithmetic means of titers of complement fixing antibodies  
in the sera of guinea pigs vaccinated with antigens from phase  
I and II Burnet rickettsiae

a) Антиген		Титр антител в сыворотках, взятых в разные дни							
b) Фаза	концентрация (в млн.)	30-й день		60-й день		на 60-й день вве- дено 100 000 ID рик- кетсия Бернета (фаза I)	80-й день		
		I фаза	II фаза	I фаза	II фаза		I фаза	II фаза	
I	1 000	80	640	20	160		640	320	
	250	80	640	40	40		1 280	640	
	62	10	320	10	40		1 280	1 280	
	31	40	160	20	40		1 280	1 280	
	16	—	40	—	20		2 560	2 560	
II	1 000	—	640	—	320		320	2 560	
	250	—	640	—	160		320	2 560	
	62	—	320	—	160		160	2 560	
	31	—	320	—	40		640	1 280	
	16	—	320	—	10		640	1 280	
I	Контроль h)						20	320	

Legend:

- a) antigen
- b) phase
- c) concentration (millions)
- d) antibody titer in sera obtained on different days
- e) ...th day
- f) ... phase
- g) on the 60th day 100,000 ID of Burnet rickettsiae (phase I) were administered
- h) control

Evaluation of immunity was made by comparing the duration of the febrile period in vaccinated and control guinea pigs given the same dose of infective material.

When immunity was tested by infection with a phase II rickettsiae no differences in temperature reactions could be demonstrated: all of the guinea pigs, regardless of phase and concentration of injected material, were found to be immune. Analogous data were obtained by Ormsbae et al (1964) following infection with phase II rickettsiae.

Different temperature reactions in guinea pigs vaccinated with phase I and II antigens were observed only when testing immunity by infection with a phase I rickettsial culture. As shown in Table 3, the guinea pigs immunized with phase I antigen, regardless of the concentration of

administered material, were immune to administration of 100,000 ID of phase I rickettsia culture. The animals vaccinated with phase II antigen were less resistant to infection with the same dosage of culture: administration even of high concentrations of antigen (one billion and 250 million bacterial cells per milliliter) provided complete immunity in no more than 50% of the animals, whereas injection of a small amount of phase I antigen (16 million bacterial cells) provided total immunity in all of the animals.

Table 3  
Degree of immunity of guinea pigs following infection with 100,000 ID of Burnet's rickettsiae (phase I)

(a) Вакцина			(f) Температурная реакция						
(b) фаза	(c) концентрация (в млн.)	(d) доза (в мл)	На 60-й день введено по 100 000 ID риккетсий Берннета (I фаза)	число дней	число свинок	к % конт- роля	среднее продолже- ние ре- акции	средние индексы	средние показатели
			(e)	(g)	(h)	(i)	(j)	(k)	
I	1 000 250 62 31 16	0,5 и 1 0,5 и 1 0,5 и 1 0,5 и 1 0,5 и 1		0 0 0 2 3	10 10 10 10 10	0 0 0 3,9 5,9	2 2	39,9° 39,6°	
II	1 000 250 62 31 16	0,5 и 1 0,5 и 1 0,5 и 1 0,5 и 1 0,5 и 1		27 32 41 38 47	10 10 10 10 10	52,9 62,7 80,4 74,5 92,1	3,4 3,6 4,6 4,2 5,2	39,7° 39,8° 40° 40° 40°	
I	Контроль			51	10	100	5,1	40°	

Legend:

a) vaccine	f) temperature reaction
b) phase	g) number of days
c) concentration (millions)	h) number of guinea pigs
d) dose (milliliters)	i) % in relation to control
e) on the 60th day 100,000 Burnet rickettsiae (phase I) were administered	j) mean duration (days)
	k) mean indices
	l) control

The findings showing greater immunogenic activity of the vaccine from phase I Burnet rickettsiae coincide with those of Ormsbee et al (1964) and are somewhat inconsistent with the studies of Brezina (1958). This author studied the immunogenicity of vaccines prepared from the yolk sacs of the second to third (phase I) and 13th passage (phase II) of the Florian strain isolated in Bratislava in experiments on guinea

pigs. He failed to note any differences in degree of immunity following infection with 100,000 ID of the Florian strain (phase I).

Evidently these results can be attributed to the fact that in the 13th passage the Florian strain did not entirely move to phase II and contained components of phase I that were not detectable serologically. It is known that with the shift from phase I to phase II antigenic properties change more rapidly than immunogenic ones. This is confirmed by the studies of Ormsbee et al (1964): by determining the density of rickettsiae in a  $\text{CaCl}_2$  solution, they found a small amount of phase I antigen in the Henzerling strain (22th egg passage) which behaved serologically like phase II antigen. For this same reason, Smadel et al (1948) were unable to demonstrate a difference between vaccines prepared from Henzerling (12th egg passage) and Dyer (second egg passage) strains.

#### Conclusions

1. Vaccine prepared from phase I Burnet rickettsiae is more immunogenic than the vaccine from phase II.

2. Following immunization of guinea pigs with phase I vaccine production of antibodies was observed at both "early" and "late" times following immunization, whereas phase II vaccine stimulated production of antibodies only at the "late" period.

In a concentration of 16 million bacterial cells per milliliter, phase I vaccine elicited production of complete immunity against 100,000 ID of Burnet rickettsiae (phase I), while phase II vaccine, even in high concentrations (one billion and 250 million) provided only partial immunity.

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